bio-protocol Preprint

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Assessment of thrombolysis efficacy in a mouse model

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Accelerating thrombolysis using a precision and clot-penetrating drug delivery strategy by nanoparticle-shelled microbubbles

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Detailed protocol

Assessment of thrombolysis efficacy in a mouse model

All procedures involving animals were approved by the Institutional Animal Care and Utilization Committee at Nanyang Technological University.

1. Prepare 6 to 8 weeks old C57/BL6J male mice.

2. Anesthetize mice with the 100 µL 10% chloral hydrate via intraperitoneal injection.

Note: This amount of chloral hydrate is sufficient to keep the animal in stable anesthesia and no pain for at least 1 hour.

3. Secure the mouse in the supine position on the small animal operating table. Remove fur on the left femoral with a small animal electric clipper.

4. Sterilize the left femoral with alcohol pad. And then use the scalpel and forceps to make incision on the left femoral.

5. Cut and open the skin and fascia, soft tissue with the scalpel, and remove soft tissue with the forceps to free the femoral vein from surrounding tissue and expose the left femoral vein.

6. Prepare a fresh stock solution of 20% $FeCl_3$ in pure water.

7. Cut and prepare the filter paper to 2 mm x 2 mm size. Soak the cut filter paper in the $FeCl_3$ solution.

8. Use a fine tip forceps and pick up a piece of filter paper saturated with $FeCl_3$ solution and place it directly on the exposed of left femoral vein, and keep it on site for 1-2 min.

9. Remove the filter paper and wash the left femoral vein thrombus with sterilized phosphate-buffered saline (PBS) for twice. Form the visible femoral vein thrombus. (this time point is defined as the thrombosis)

10. Divide the mice into four groups.

11. Prepare saline solution and the sample (native tPA, SiO_2-tPA, and MMB-SiO_2-tPA) at the equivalent concentration of tPA (10 µg/ml).

12. Draw sample solution using 1 ml syringe and then inject sample into the mouse via intravenously injection.

13. Attach the magnet on the left femoral vein thrombus of mouse for 25 min in the MMB-SiO_2-tPA treated group.

14. Apply the ultrasound with the intensity of 0.2 bar for 5 min in the MMB-SiO_2-tPA treated group.

15. Record the thrombolysis photos every 3 hours for 12 hours by mobile phone.

16. Euthanize mouse by CO_2 asphyxiation and cervical vertebra dislocation or any method approved by the Institute's animal research committee.

17. Cut and excise the left femoral vein from the surrounding tissue.

18. Collect the left femoral vein tissue in 4% paraformaldehyde for 24 hours, and then frozen and stain with hematoxylin-eosin.

19. Analyze the thrombolytic efficiency from the section photos using Image J software by an investigator blinded to the treatment.

1. Click "**File**" and "**Open**" to select the image file to be processed. After the image is imported, press the "**Analyze**" and "**Set Scale**". Draw a black line with the same length as the ruler. And enter the length of the ruler.

2. Press "**Freehand Selections**" and draw the area in the image. Complete all the measurements, then click on "**Analyze**" | "**Measure**".

3. Determine the thrombolytic efficiency by the area ratio of vascular occlusion to total vasculature.

Assessment nanoparticle penetration in a mouse model

1. Prepare 6 to 8 weeks old C57/BL6J male mice.

2. Anesthetize mice with the 100 µL 10% chloral hydrate via intraperitoneal injection.

Note: This amount of chloral hydrate is sufficient to keep the animal in stable anesthesia and no pain for at least 1 hour.

3. Secure the mouse in the supine position on the small animal operating table. Remove fur on the left femoral with a small animal electric clipper.

4. Sterilize the left femoral with alcohol pad. And then use the scalpel and forceps to make incision on the left femoral.

5. Cut and open the skin and fascia, soft tissue with the scalpel, and remove soft tissue with the forceps to free the femoral vein from surrounding tissue and expose the left femoral vein.

6. Prepare a fresh stock solution of 20% $FeCl_3$ in pure water.

7. Cut and prepare the filter paper to 2 mm x 2 mm size. Soak the cut filter paper in the $FeCl_3$ solution.

8. Use a fine tip forceps and pick up a piece of filter paper saturated with $FeCl_3$ solution and place it directly on the left femoral vein thrombus, and keep it on site for 1-2 min.

9. Remove the filter paper and wash the left femoral vein thrombus with sterilized phosphate-buffered saline (PBS) for twice.

10. Divide the mice into two groups.

11. Prepare MMB-SiO_2-tPA stock solution sample.

12. Draw sample solution using 1 ml syringe and then inject 100 µL sample into the mouse via intravenously injection.

13. Attach the magnet on the left femoral vein thrombus of mouse for 25 min in all group.

14. Apply the ultrasound with the intensity of 0.2 bar for 5 min in the one group, and without any treatment in another group.

15. Euthanize mouse after treatment by CO_2 asphyxiation and cervical vertebra dislocation or any method approved by the Institute's animal research committee.

16. Cut and excise the left femoral vein thrombus from the surrounding tissue.

17. Collect the left femoral vein tissue in 4% paraformaldehyde for 24 hours, and then frozen and stain with hematoxylin-eosin.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Gao, Y. and Wang, S. y.(2021). Assessment of thrombolysis efficacy in a mouse model. Bio-protocol Preprint. bio-protocol.org/prep816.
2. Wang, S., Guo, X., Xiu, W., Liu, Y., Ren, L., Xiao, H., Yang, F., Gao, Y., Xu, C. and Wang, L.(2020). Accelerating thrombolysis using a precision and clot-penetrating drug delivery strategy by nanoparticle-shelled microbubbles. Science Advances 6(31). DOI: 10.1126/sciadv.aaz8204